



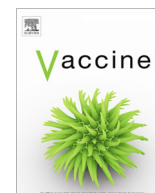
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A Brighton Collaboration standardized template with key considerations for a benefit/risk assessment for an inactivated viral vaccine against Chikungunya virus

Libia Milena Hernandez^a, K. Sumathy^b, Sushant Sahastrabuddhe^a, Jean-Louis Excler^a, Sonali Kochhar^{c,e}, Emily R. Smith^{d,*}, Marc Gurwith^d, Robert T. Chen^d,
For the Benefit-Risk Assessment of Vaccines by Technology Working Group (BRAVATO, ex-V3SWG)¹

^a International Vaccine Institute (IVI), Seoul, Republic of Korea^b Bharat Biotech International Limited (BBIL), Hyderabad, Telangana, India^c Global Healthcare Consulting, New Delhi, India^d Brighton Collaboration, A Program of the Task Force for Global Health, Decatur, GA, USA^e University of Washington, Seattle, USA

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ABSTRACT

Inactivated viral vaccines have long been used in humans for diseases of global health threat (e.g., poliomyelitis and pandemic and seasonal influenza) and the technology of inactivation has more recently been used for emerging diseases such as West Nile, Chikungunya, Ross River, SARS and especially for COVID-19.

The Brighton Collaboration Benefit-Risk Assessment of Vaccines by Technology (BRAVATO) Working Group has prepared standardized templates to describe the key considerations for the benefit and risk of several vaccine platform technologies, including inactivated viral vaccines. This paper uses the BRAVATO inactivated virus vaccine template to review the features of an inactivated whole chikungunya virus (CHIKV) vaccine that has been evaluated in several preclinical studies and clinical trials.

The inactivated whole CHIKV vaccine was cultured on Vero cells and inactivated by β -propiolactone. This provides an effective, flexible system for high-yield manufacturing. The inactivated whole CHIKV vaccine has favorable thermostability profiles, compatible with vaccine supply chains.

Safety data are compiled in the current inactivated whole CHIKV vaccine safety database with unblinded data from the ongoing studies: 850 participants from phase II study (parts A and B) outside of India, and 600 participants from ongoing phase II study in India, and completed phase I clinical studies for 60 subjects. Overall, the inactivated whole CHIKV vaccine has been well tolerated, with no significant safety issues identified. Evaluation of the inactivated whole CHIKV vaccine is continuing, with 1410 participants vaccinated as of 20 April 2022. Extensive evaluation of immunogenicity in humans shows strong, durable humoral immune responses.

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1. Introduction

The Brighton Collaboration (<https://www.brightoncollaboration.org>) was launched in 2000 to improve the science of vaccine safety [1]. The Brighton Viral Vector Vaccine Safety Working Group (V3SWG) was formed in 2008 in recognition of the increasing importance of viral vectors for the development of new vaccines

and the need to understand their associated safety issues [2]. To better meet the needs of many other platform technologies used to develop vaccines to prevent COVID-19 beyond vaccines based upon viral vectors, the V3SWG was renamed the Benefit-Risk Assessment of Vaccines by Technology (BRAVATO) Working Group in July 2020. BRAVATO has prepared standardized templates to describe the key considerations for the benefit and risk of several vaccine platform technologies, including inactivated viral vaccines, to facilitate scientific discourse among key stakeholders [2]. This paper uses the BRAVATO inactivated virus vaccine template to review the features of an inactivated whole chikungunya virus

* Corresponding author.

E-mail address: bc-coordinator@taskforce.org (E.R. Smith).¹ See Acknowledgement for other BRAVATO members.

(CHIKV) vaccine that has been evaluated in several preclinical studies and clinical trials (see Table 1).

2. Background

Chikungunya virus (CHIKV) is an Alphavirus of the family Togaviridae. It is an arthropod-borne virus transmitted by mosquitoes. The spherical virus consists of a viral envelope and a nucleocapsid. The viral genome consists of a single positive-strand RNA that encodes two polyproteins; the non-structural polyprotein consists of nsP1, nsP2, nsP3 and nsP4 proteins involved in viral replication, and the structural polyprotein consists of E3, E2, 6K proteins that form the viral capsid and the E1 protein of the viral envelope. Alphaviruses have conserved domains at the 5' and 3' ends of the genome as well as in the intergenic region that play an important role in the regulation of viral RNA synthesis. The E1 and E2 glycoproteins form heterodimers which associate as trimeric spikes on the viral surface. CHIKV attaches to the cells through the trimeric spikes which serves as major antigenic determinants of the virus.

CHIKV most likely originated in East and central Africa where the virus is endemic, existing in a sylvatic cycle between mosquitoes and nonhuman primates living in forests. The virus is maintained in a complex sylvatic and rural cycle, progressing to an urban cycle approximately every 5 to 20 years, causing global pandemics.

The sylvatic cycles of Chikungunya virus exist primarily in Africa between non-human primates, rodents and possibly in bats and forest dwelling *Aedes* species (*Ae. albopictus*, *Ae. fuscifer*, *Ae. africanus*, *Ae. taylori*). The rural transmission occurs when rural population bitten by infected forest-dwelling mosquitoes, especially by *Ae. albopictus*. Movement of infected humans can result in establishment of a large urban transmission through urban *Aedes* mosquitoes (*Ae. aegypti* and *Ae. albopictus*), where a human-mosquito-human cycle is established. *Ae. albopictus* feeds on a range of hosts including humans and wild mammals whereas *Ae. aegypti* primarily feeds on human, and most forest dwelling *Aedes* species (*Ae. fuscifer*, *Ae. africanus*, *Ae. taylori*) feeds primarily on animals.

In Asia, Europe, the Americas the CHIKV seems to be maintained in an urban cycle with *Ae. aegypti* and *Ae. albopictus* mosquito vectors, whereas CHIKV transmission in Africa involves a sylvatic cycle, primarily with *Ae. fuscifer* and *Ae. africanus* mosquitoes.

After human CHIKV infection, the incubation period can vary from 1 to 12 days (average, 2–7 days). Infection causes high levels of viremia, which usually last for 4–6 days after the onset of symptoms. CHIKV infection is symptomatic in most children and adults who are infected, with less than 15% having asymptomatic seroconversion.

CHIKV is transmitted to people through bites from infected *Ae. aegypti* and *Ae. albopictus* mosquitoes [3]. These mosquitoes breed in or near human habitations and prefer to feed on humans during the daytime in shady areas and early in the evening. Horizontal transmission in *Aedes* spp can occur and contribute to the maintenance of CHIKV cycles. Vertical transmission is rare but has been observed under natural and experimental conditions. In Africa, CHIKV circulates primarily in an enzootic cycle, with occasional spillover infections of humans.

CHIKV typically causes a self-limiting febrile illness, chikungunya fever (CHIKF), characterized by chronic, severe joint pain, and sometimes accompanied by an itchy maculo-papular skin rash. Severe complications, such as encephalitis, may occur in the elderly and in individuals with comorbidities, and peripartum infections can be fatal or involve severe neurologic sequelae in fetuses and infants [4,5]. CHIKV is enzootic in Africa, where trans-

mission involves different arboreal *Aedes* spp. vectors and nonhuman primates (NHP) in forested habitats. Direct spillover infections of humans from these enzootic cycles probably occur in many regions of Sub-Saharan Africa. In Asia, CHIKV is endemic and causes recurrent and sometimes large epidemics, especially in the Indian subcontinent and in Southeast Asia [4–6].

In 2004, CHIKV reemerged to cause large outbreaks, which began on the coast of Kenya and ravaged several Indian Ocean islands and the Indian subcontinent in the years 2005 to 2006, before spreading to initiate transmission in Southeast Asia [7,8]. A few years later, CHIKF outbreaks were also reported in the Arabian Peninsula [9]. In 2013, autochthonous (locally originating) chains of transmission of CHIKV were identified for the first time in the Americas [10]. CHIKV has expanded its range of activity to include temperate regions in part by adapting for transmission by *A. albopictus*, including two outbreaks in Italy in 2007 and 2017 [11,12] and two in France. The unabated spread and increasing burden of CHIKF underscores the need to develop an effective vaccine [13].

Vaccines for CHIKF have been developed for several decades, and comprehensive reviews of these have been published in recent years [14–16].

2.1. Inactivated whole virion CHIKV vaccine development

Vaccination is the most cost-effective option for protecting the at-risk population from CHIKV infection. Inactivated viral vaccines have long been used in humans for diseases of global health threat, including poliomyelitis, pandemic and seasonal influenza, rabies, hepatitis A, Japanese encephalitis and tick borne encephalitis [17], and the technology of inactivation has more recently been used for emerging diseases such as West Nile, Chikungunya, Ross River, SARS [18,19] and especially for COVID-19 (SARS-CoV-2) [20].

The potential advantages of inactivated vaccines are that they cannot replicate in the host or revert to pathogenicity and are non-transmissible [17]. Whole inactivated virus particles have the potential to induce a broad range of both humoral and cellular responses against all the different epitopes presented by the virus.

Data obtained so far in extensive preclinical testing in several animal models have clearly shown that neutralizing antibodies protect against the control of CHIKV infection post-vaccination. PRNT₅₀ neutralizing antibody titers in human CHIKV convalescent sera vary from 40 to 5120, comparable to previously reported titers [21]. The presence of IgG3 antibodies has been suggested to correlate with viral clearance and long-term protection against Chikungunya infection [22,23]. These studies provide convincing evidence that levels of neutralizing antibodies are strongly correlated with protective immune response.

3. Disclaimer

The findings, opinions, conclusions, and assertions contained in this consensus document are those of the individual members of the Working Group. They do not necessarily represent the official positions of any participant's organization (e.g., government, university, or corporations) and should not be construed to represent any Agency determination or policy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 1
Brighton Collaboration: Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines.

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
1. Authorship and Affiliation	Information	
1.1 Author(s) and affiliation (s)	Libia Milena Hernandez Medina from International Vaccine Institute (IVI) Sumathy Kandaswamy from Bharat Biotech International Limited (BBIL) July 19th 2021	
1.2 Date completed/updated		
2. Basic Vaccine information	Information	Comments/Concerns
2.1 Vaccine name	(BBV87. Purified, Inactivated Chikungunya Virus Vaccine (Adsorbed).	
2.2 Virus name, genus, family, strains/serotypes, origin (e.g., geography, patient, asymptomatic), and any other specific characteristics such as genetic modifications	Chikungunya virus is of the genus, Alphavirus, family Togaviridae. The vaccine strain designated as CHIK/03/06 belongs to the Indian Ocean Lineage of the East, Central, South African (ECSA) genotype (24). It was isolated from blood sample of a Chikungunya virus infected patient in Hyderabad India, in 2006 during an epidemic outbreak. The patient had symptomatic Chikungunya virus infection with high fever and joint pains. No genetic modifications were done to the virus isolate [25]	The Chikungunya Virus: Phylogenetic analysis has shown 4 different genotypes of CHIKV based on geographic regions: the west African genotype (Senegal and Nigeria), the ECSA genotype, the Asian genotype, and the Indian Ocean Lineage (IOL) genotype.
2.3 Substrate for vaccine virus growth (e.g., cell substrate, eggs, animal, etc.)	Grown on Vero cells.	
2.4 Inactivation method	Beta propiolactone (BPL) inactivated	
2.5 Adjuvant (if applicable)	Aluminum hydroxide	
2.6 Final vaccine formulation components	Single human dose (SHD) contains: - Purified, inactivated Chikungunya virus antigen - 20 µg or 40 µg - Aluminum as aluminum hydroxide I.P.: 0.25 mg - 2-Phenoxyethanol, USP (preservative) 2.5 mg - Phosphate buffered saline: q.s. to 0.5 mL	
2.7 Route and method of delivery (e.g., intramuscular injection, microneedles, skin patch, intranasal, other mucosal)	Intramuscular injection	
3. Target Pathogen and Population	Information	Comments/Concerns
3.1 What is the target pathogen?	Chikungunya Virus	
3.2 What are the disease manifestations caused by the target pathogen in humans, for the following categories: ● In healthy people	The most frequent symptoms are: High fever, very sharp onset of crippling joint pains (polyarthralgia*), polyarthrititis, maculo-papular rash, myalgia and edema in hand and feet. These symptoms can be severe and disabling, causing much morbidity. Special considerations: can evolve to chronic arthropathy. * Disabling polyarthralgia is a key symptom for differential diagnosis with a positive predictive value greater than 80%	
● In immunocompromised people	<u>Mortality:</u> Although morbidity is debilitating, CHIKV mortality rates are low. An increase in mortality has been described in some outbreaks, probably as a result of neurologic disorders mainly in neonates, immunocompromised patients, and the elderly. In Europe, the case fatality rate was 2.5 per 1000 clinical cases, lower than that reported in the 2007 outbreak in Italy (0.5%) but consistent with that reported from Reunion Island (1 death per 1000 clinical cases)[26–28,31]. As with most viral illnesses, people at a higher risk for more severe disease include immunocompromised people. An increase in mortality was observed in the last epidemics, probably as a result of neurologic disorders mainly in neonates, immunocompromised patients, and the elderly [26–28]	

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Table 1 (continued)

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines	
● In neonates, infants, children	<p>In infected newborns, symptoms generally develop on days 3 to 7 of life with fever, rash, and peripheral edema. Pathology typically reveals a bicytopenia, increased prothrombin time, and aspartate aminotransferase level. Neonatal symptoms range from mild presentation (43%) to severe infection with encephalitis (53%) that requires intensive care.</p> <p>Fever and acute respiratory distress have also been reported. Neurologic complications can have severe effects on postnatal neurologic development, such as lower development quotient at age of 2 years, and moderate to severe global neurodevelopmental delay. Complication in infants: The presentation in infants can be complicated by seizures, hemorrhagic syndrome, hemodynamic disorders, and myocardial dysfunction [26–28].</p>
● During pregnancy and in the fetus	<p>Severe disease occurs in neonates exposed during pregnancy. Mother-to-child chikungunya virus transmission is frequent in the context of intrapartum maternal viremia, and often leads to severe neonatal infection. Intrapartum contamination without actual placental infection has been well documented for CHIKV virus, which is not able to infect the placenta. CHIKV is not transmitted to the fetus in the absence of placental breaches, which allow transfer of maternal blood to the fetal circulation. Chikungunya represents a substantial risk for neonates born to viremic pregnant women [32–33].</p>
● In elderly	<p>Severe disease can occur in the elderly and an increase in mortality has been observed in the last outbreaks, probably as a result of neurologic disorders [26].</p>
● In any other special populations	<p>Populations with comorbid illnesses such as diabetes, heart disease, chronic liver and kidney disease, and human immunodeficiency virus are at risk to present complications [26].</p>
3.3 Briefly, what are the key epidemiologic characteristics of the disease caused by the target pathogen (e.g., incubation period, communicable period, route/s of transmission, case fatality rate, transmissibility characteristics such as basic reproductive ratio (R_0), and spontaneous mutation)?	<p>CHIKV most likely originated in East and central Africa where the virus is endemic as a sylvatic cycle between mosquitoes and nonhuman primates living in forests. The virus is maintained in a complex sylvatic and rural cycle, progressing to an urban cycle approximately every 5 to 20 years, causing global pandemics.</p> <p><u>Transmission cycles</u></p> <p>The sylvatic cycles of Chikungunya virus exist primarily in Africa between non-human primates, rodents and possibly in bats and forest-dwelling Aedes species (<i>Ae. albopictus</i>, <i>Ae. furcifer</i>, <i>Ae. africanus</i>, <i>Ae. taylori</i>). The rural transmission occurs when rural population is bitten by infected forest-dwelling mosquitoes, especially by <i>Ae. albopictus</i>.</p> <p><u>Movement</u></p> <p>of infected humans can result in establishment of a large urban transmission through urban Aedes mosquitoes (<i>Ae. aegypti</i> and <i>Ae. albopictus</i>), where a human-mosquito-human cycle is established. <i>Ae. albopictus</i> feeds on a range of hosts including humans and wild mammals whereas <i>Ae. aegypti</i> primarily feeds on human, and most forest dwelling Aedes species (<i>Ae. furcifer</i>, <i>Ae. africanus</i>, <i>Ae. taylori</i>) feeds primarily on animals.</p> <p>These different geographic genotypes exhibit differences in their transmission cycles. In Asia, Europe, the Americas the CHIKV seems to be maintained in an urban cycle with <i>Ae. aegypti</i> and <i>Ae. albopictus</i> mosquito vectors, whereas CHIKV transmission in Africa involves a</p>

Table 1 (continued)

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
	<p>sylvatic cycle, primarily with <i>Ae. furcifer</i> and <i>Ae. africanus</i> mosquitoes.</p> <p>The incubation period can vary from 1 to 12 days (average, 2–7 days). CHIKV infection causes high levels of viremia, which usually last for 4 to 6 days after the onset of symptoms. CHIKV infection is symptomatic in most children and adults who are infected, with less than 15% having asymptomatic seroconversion.</p> <p>CHIKV is transmitted to people through bites from infected <i>Ae. aegypti</i> and <i>Ae. albopictus</i> mosquitoes. These mosquitoes breed in or near human habitations and prefer to feed on humans during the daytime in shady areas and early in the evening. Horizontal transmission in <i>Aedes</i> spp can occur and contribute to the maintenance of CHIKV cycles. Vertical transmission is rare but has been observed under natural and experimental conditions. As mentioned above, In Africa, CHIKV circulates primarily in an enzootic cycle, with occasional spillover infections of humans [26].</p> <p>As with most viral illnesses, people at a higher risk for more severe disease include the newborn, the elderly, and those with comorbid illnesses such as diabetes, heart disease, chronic liver and kidney disease, and human immunodeficiency virus infection 26–28.</p> <p>Chikungunya virus-specific antibodies have been shown to play an important role in the protection against CHIKV [29]. Natural infection confers long term protective immunity that could last up to two decades after infection [40].</p> <p>The passive transfer of neutralizing monoclonal antibodies has efficiently prevented CHIKV infection or disease symptoms in mice and nonhuman primates.</p> <p>In a recent cohort in the Philippines, a PRNT80 titer $\geq 1:10$ was associated with zero relative risk of symptomatic CHIKV disease, and 0.018 risk of subclinical seroconversion. This suggests that the presence of detectable CHIKV neutralizing antibodies correlates with decreased risk of both symptomatic CHIKV and subclinical seroconversion [35,36]</p> <p>The role of cellular responses during CHIKV infection is multifaceted and remains partially unclear. CD4 T cells are crucial to promote antibody responses but are also implicated in CHIKV-mediated joint immunopathology. CD8 T cells, NK cells and gamma-delta ($\gamma\delta$) T cells have all been suggested to contribute to CHIKV immunity, but the degree of their involvement in protection in humans remains a matter of debate [37]</p> <p>The impact of CHIKV in terms of burden of disease, work and school absenteeism, and other financial costs is particularly high, especially given its formidable epidemic potential.</p> <p>The capacity of CHIKV to adapt to a new mosquito vector has been demonstrated during the Indian Ocean epidemic, when a series of mutations increased fitness for transmission by <i>Ae. albopictus</i>, that can survive at higher latitudes than <i>Ae. aegypti</i>. This may lead to the occurrence of outbreaks in temperate climates, as seen in Europe.</p>	
3.4 What sections of the population are most affected by the target pathogen (e.g., pediatric, pregnant, lactating women (breast feeding), adult, elderly)		
3.5 What is known about the immune responses, duration, and potential correlates of protective immunity to the target pathogen or to the disease?		
3.6 Please describe any other key information about the target pathogen or population that may inform benefit-risk		
4. Characteristics of Antigen	Information	Comments/ Concerns
4.1 Virus strains, sequence (including homology among strains), source, propagation, disruption, whole virus or subunit/subvirion (if	The candidate inactivated (beta propiolactone) whole virion vaccine, BBV87 was developed using the strain CHIK/03/06, an Indian (2006)	

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Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
applicable)?	isolate of the East, Central, South African (ECSA) genotype [24].	
4.2 Is the vaccine likely to induce immunity to all strains/genotypes of the target pathogen? What is the evidence?	Vaccine antisera cross neutralized various strains of the ECSA genotype isolated from 2004 to 2013 (unpublished data).	
4.3 What is known about the immune response to the vaccine in animals and/or humans (binding, neutralizing antibody, functional, and, B-cell, T-cell memory, etc.)?	BBV87 elicited high level of neutralizing and binding antibodies in Balb/c mice. In a dose ranging study, neutralizing antibody titers were estimated by 50% plaque reduction neutralization test (PRNT ₅₀) and the log ₁₀ PRNT ₅₀ titers ranged from 2.52 to 3.28 for 20 µg/dose and was 2.94 to 3.91 for 40 µg dose strength (unpublished). Passive antibody transfer studies conferred complete protection against challenge with 10 ⁵ pfu of the virus.	
	In the first phase I study, either 10 µg, 20 µg or 30 µg of the vaccine per single human dose (SHD) was administered in three doses 28 days apart. There was 100% seroconversion after 2nd and 3rd dose across all dose strengths. In a second phase I study, two doses of 40 µg were administered 28 days apart, 100% seroconversion was achieved after single dose of 40 µg. The PRNT ₅₀ titers remained high at the end of 6 months after the last dose administration.	
5. Inactivation Method(s)	Information	Comments/ Concerns
5.1 Method/s (e.g., thermal, beta propiolactone, UV, formaldehyde, ionizing radiation) and potential impact on safety	Beta propiolactone (BPL)	
5.2 At what stage of the downstream process is inactivation/s performed and why?	BPL Inactivation is performed on clarified virus harvest. BPL is removed in subsequent purification steps.	
5.3 QC/confirmation method/log reduction in viability	Residual inactivated virus if any, is enumerated in a plaque assay in virus inactivation test (VIT) and by virus amplification test (VAT) In the VAT assay, the inactivated virus sample is amplified three times in vitro, and the supernatant from the third in vitro culture is checked for residual live virus if any, by plaque assay.	
5.4 Could the inactivation method/s compromise the antigenic structure of the vaccine (e.g., conformation of the protein antigens)	Inactivation conditions were optimized for concentration, temperature, and duration of inactivation for minimum exposure to BPL. Inactivated vaccine elicited good titers of neutralizing antibodies indicating preservation of critical conformational epitopes on the vaccine antigen.	
6. Adjuvant (optional, if applicable)	Information	Comments/ Concerns
6.1 Describe the type of adjuvant, if it has been tested in humans, whether novel or commercialized, and if applicable, what other vaccines (preventive and therapeutic) are formulated with this adjuvant	Aluminum hydroxide from Brenntag, Essen Germany is used. Aluminum containing compounds such as aluminum hydroxide, aluminum phosphate and amorphous aluminum sulphate phosphates are widely used as vaccine adjuvants. Several vaccines with aluminum containing compounds have been commercialized, such as vaccines for Japanese encephalitis virus, HPV, hepatitis A, hepatitis B and acellular pertussis vaccines to name a few.	
6.2 What is the evidence that an adjuvant improves/boosts/enhances the immune response?	Inactivated antigen adsorbed to aluminum hydroxide elicited higher titer of neutralizing antibodies. Formulation with aluminum hydroxide also enabled vaccine storage at 2–8 °C.	
6.3 What is the mechanism of action of the adjuvant (if known)?	Aluminum hydroxide is a well-studied adjuvant used in human vaccines for several decades. Binding with aluminum hydroxide provides a 'depot' effect as the antigen is released slowly from the formulation for uptake by macrophages. It has been shown that aluminum hydroxide adjuvant activates innate immune responses mediated by NLRP3 inflammasome pathway, which in turn could modulate the humoral adaptive immune responses.	
6.4 How is the adjuvant formulated with the antigen?	Binding of the purified inactivated bulk with aluminum hydroxide is	

Table 1 (continued)

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
6.5 How might the adjuvant impact the safety profile of the vaccine?	done in sterile vessels under optimized conditions to ensure complete binding. Aluminum hydroxide as vaccine adjuvant did not adversely affect the safety profile of the candidate vaccine as demonstrated in the GLP pre-clinical toxicity studies and in the Phase I clinical trials [24].	
6.6 Summarize the safety findings (preclinical and clinical) with the adjuvant, formulated with any antigen	In pre-clinical GLP toxicity studies with the adjuvanted CHIKV vaccine, episodic administration of 5 vaccine doses up to 5 to 10 times the dose strength administered in phase I clinical trials, did not demonstrate any local or systemic toxicity. No adverse biochemical, physiological, or histopathological changes were observed in all organs including immune organs [24,34]. Three doses of either 10, 20 or 30 µg /0.5 mL/dose of vaccine when administered intramuscularly in humans in phase I clinical trials (n = 60, 15 per dose group and 15 in placebo group) did not elicit any clinically significant out of range values in hematological, renal and hepatic function tests. In the vaccine group, 45 adverse events were reported. Most of the adverse events were mild, and all the AEs resolved without any sequelae. Thus, BBV87 was found to be safe and well tolerated in healthy adults of 18 to 50 years age [34]. In the second phase I study, 40 µg/SHD was administered in two doses, 28 days apart (n = 20; 15 in vaccine group and 5 in placebo group). 8 solicited adverse events were reported. Of the 8 adverse events, 7 adverse events were mild and 1 was moderate in intensity. All AEs resolved within 1–3 days without any sequelae. In both the phase I studies, no Serious Adverse Event and unsolicited adverse events were reported in the follow-up period of six months post vaccination with second dose.	
7. Delivery and Administration	Information	Comments/ Concerns
7.1 Describe how the mode of vaccine delivery may impact safety (e.g., intramuscular by needle injection, microneedles, intranasal, oral, or combination thereof)	Intramuscular by needle injection. The local solicited adverse events are pain, tenderness, erythema/redness and induration/swelling	
7.2 If the vaccine is part of a heterologous prime-boost regimen, describe the regimen that this vaccine is a part of and the possible impact on safety	The same vaccine is administered in two doses, 28 days apart in a prime-boost regimen	
8. Toxicology and Nonclinical	Information	Comments/ Concerns
8.1 What is the possible risk of autoimmunity or a harmful immune response?	Specific risk of autoimmunity has not been assessed. In GLP repeated dose toxicity studies in rats and rabbits, administration of 5 vaccine doses did not reveal any adverse findings [24]. There was no evidence of autoimmunity or adverse immune reactions in phase I subjects who were followed up for 6 months after last vaccine dose administration [34].	
8.2 Summarize the preclinical safety data that supports the use of this product in humans including any related information from similar products	Immune response in GLP repeated dose toxicity studies with 5 episodic doses of the vaccine 5 to 10 times the dose strength administered in phase I clinical trials did not show any adverse safety profile. GLP non-clinical toxicity studies such maximum tolerated dose, local tolerance, acute toxicity and repeated dose toxicity studies in rats and rabbits were designed to obtain broad spectrum of information on potential toxicity of the investigational vaccine such as local inflammatory reactions, effect on the draining lymph nodes and the immune system, and systemic toxicity. Daily in-life parameters, clinical	

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Table 1 (continued)

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
	<p>observations, weekly body weights and food consumption were recorded. Hematology and serum chemistries were studied following the first dose and after the last dose administration, and at the end of the recovery period in the recovery group and included an evaluation of relative and absolute differential white blood cell counts, albumin/globulin ratio, enzymes, electrolytes, and coagulation parameters. Urinalysis was included. At study termination, final body weights were obtained, and serum chemistry, hematology and immunological investigation were performed in terminal blood samples. Immune response induced by the vaccine candidate confirmed the choice of the relevant animal model. Histopathological evaluations included tissues of the immune organs, i.e. axillary and mesenteric lymph nodes, thymus, spleen, bone marrow and Peyer's patches, and pivotal organs such as brain, kidneys, liver, heart, reproductive organs and the site of vaccine administration. Considering that Chikungunya virus causes arthritic symptoms, tibia-femoral joint tissues and skeletal muscles were also included for histopathological evaluation.</p> <p>The candidate vaccine, BBV87 did not demonstrate any effects on biochemical, hematological, physiological parameters indicative of toxicity. Histopathology of the immune and other highly perfused organs, as well as target organs for potential arthritogenic effect such as femur-tibia joints and skeletal muscles indicated no adverse or arthritogenic effect of the vaccine in short and long term in animals.[24]</p> <p>In GLP developmental and reproductive toxicity study in rabbits, repeated intramuscular injection of the highest dose used in phase I clinical trials was well tolerated, and did not produce adverse effects on female reproductive performance, fetal development or post-natal growth and development of F1 offspring. [41]</p> <p>In dose ranging study, neutralizing antibody titers in vaccinated mice were estimated by 50% plaque reduction neutralization test (PRNT₅₀). The log₁₀ PRNT₅₀ titers ranged from 2.52 to 3.28 for 20 µg/dose and was 2.94 to 3.91 for 40 µg dose strength, that are used in ongoing phase II studies and conferred complete protection from virus challenge. Passive antibody transfer of BBV87 vaccine antisera protected against challenge with 10⁵ pfu of the virus (unpublished).</p> <p>*Additional information in recent NHP studies is described in section 9.1. No evidence of disease enhancement was detected in Balb/c mice in virus challenge studies after vaccination with two or three doses.</p> <p>Although the effect of this vaccine has not been studied on activation of innate immunity, the vaccine is not expected to elicit adverse effects as evidenced by pre-clinical and clinical safety data so far.</p>	
8.3 Summarize the preclinical immunogenicity and efficacy data that supports the use of this product in humans including any related information from similar products		
8.4 What is the evidence of disease enhancement or absence thereof in vitro or in animal models?[13]		
8.5 Would the vaccine in its final formulation have any impact on innate immunity? If so, what are the implications for benefit-risk?		
9. Human Efficacy and Other Important Information	Information	Comments/ Concerns
9.1 What is the evidence that the vaccine would generate a protective immune response in humans (e.g., natural history, passive immunization, animal challenge studies)?	Two 20 µg doses of the vaccine candidate BBV87 administered intramuscularly to Cynomolgus macaques (N = 5) 28 days apart induced a robust anti-CHIKV ELISA binding IgG response 28 days after the second dose. Vaccination also provided complete protection against viremia (as assessed by qRT-PCR) as compared to placebo group upon challenge with 1x10e5 PFU CHKV administered intradermally. Similarly, the day-night temperature pattern observed before challenge remained regular in vaccinated animals but was disrupted in the placebo group 24 to 72 h post challenge.	

Table 1 (continued)

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
9.2 Describe other key information that may impact benefit-risk	<p>This global impact of CHIKV is constantly growing, due to the introduction and spread of the virus into new continents in which it finds optimal conditions for its expansion, including a completely naïve population and a presence of both vectors that may lead the occurrence of outbreaks in different climates.</p> <p>Establishing a correlate of immune protection based on neutralizing antibody levels is a valuable option to accelerate the development of this important vaccine.</p> <p>The identification of immunological endpoint(s), that are reasonably likely to predict a clinical benefit, is an alternative being used for vaccine development and it is the approach that adopted with the clinical studies.^[30]</p>	
10. Adverse Event (AE) Assessment of the Vaccine Platform (*see Instructions):	Information	Comments/ Concerns
10.1 Approximately how many humans have received this vaccine to date? If variants of the vaccine, please list separately.	<p>Phase I studies in India:</p> <p>Phase I 10 µg, 20 µg and 30 µg (Dose escalation): 45 subjects (15 in each dose).</p> <p>Phase I 40 µg: 15 subjects</p> <p>The seamless phase II and III studies in India and Latin America and Asia (1600 and 3210 participants respectively) is ongoing, and will enroll a total of 3,210 participants distributed in 3 parts as follows: The study in Latin America and Asia have recruited and vaccinated 450 participants testing the 40 µg in phase II with a randomization ratio 2:1 with 2 treatment groups (i.e., Group 1: test vaccine with 40 µg of BBV87 and Group 2: placebo).</p> <p>In addition to this, phase II part B which is a dose selection between 20 µg and 40 µg BBV87 enrolled 400 participants to receive either of 20 µg, 40 µg or placebo randomized at 2:2:1 ratio and given at 0–28 days interval. Phase III (Part C) is to assess immunogenicity and safety of the selected dose from Part B. A total of 2,360 participants will be included. Part C will only be randomized for immunogenicity sub cohort of 360 participants at 3:1 ratio, to receive either the selected dose from Part B or placebo. The remaining 2,000 participants will be part of the safety cohort and will receive BBV87 vaccine at the selected dose (i.e., study vaccine).</p> <p>The study in India included 600 participants in phase II and 1000 participants in phase III</p> <p>1. Safety Monitoring Procedures: IVI has a Safety Monitoring Committee (SMC) that meets and reviews safety data on a regular basis. Any safety signal will promptly be discussed with the Study safety monitor, the clinical team and ad hoc DSMB meeting will be performed, if required and the site PI will subsequently be informed of the results of safety evaluation as soon as available.</p> <p>There are charters for SMC and DSMB in which these procedures are documented.</p> <p>IVI is also setting up its own Pharmacovigilance system and a PV officer will assess all safety information from all studies as well as putting in a place early signal detection.</p>	
10.2 Method(s) used for safety monitoring:		

(continued on next page)

Table 1 (continued)

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
	<p>2. For this reason, in the protocol, the stopping rules are formulated for part A only since all parts will be closely monitored for safety. Safety monitoring will continue in parts B (phase II) and C (phase III). In Part A, the DSMB reviewed the safety data from the initial 90 participants 7 days post first dose prior to proceeding to the second component enrollment. In part B, the data collected from D0 to one month post dose 2 (8 weeks PD1), will be reviewed by the DSMB and will provide recommendation for go/no go decision to proceed to Part C.</p> <p>3. Throughout the study, all the CHIK suspected cases that fulfill the definition of WHO [38], will be brought to the research center for medical care and for clinical laboratory testing, including a RT-PCR to confirm CHIK cases and will be reported as an AESI. If the case is CHIK negative and the adverse event such as joint pain or fever started during the notification period (28 days PD), then this will be reported as an adverse event.</p> <p>The WHO case definition followed in the study includes fever greater than 38.5 °C and joint pain (usually incapacitating) with acute onset; and with epidemiological criterion (i.e., resident or visitor in areas with local transmission of chikungunya for the last 15 days).</p>	
● Spontaneous reports/passive surveillance	Yes	Spontaneous reports, active surveillance by a call during the first 3 days post each dose, diary card the first 7 days post each dose and passive surveillance
● Diary	Yes	If yes, number of days: 7
● Other active surveillance	Yes	If yes, describe method (e.g., LTFU) and list the AEs solicited: The solicited AEs list is as follows: ● Local reactions at the site of injection: pain, tenderness, erythema/redness and induration/swelling. ● Systemic reactions (adapted by age strata): fever, nausea, vomiting, arthralgia, chills, headache, fatigue, myalgia, and rash. In addition to this, in Latin America and Asia study there will be a long-term safety follow- up visit at month 6 post last dose.
10.3 What criteria were used for grading the AEs?		
● 2007 US FDA Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials	Yes: It is used for the Phase II/III Adaptive Seamless Design, Randomized, Controlled Trial to Evaluate Safety and Immunogenicity of 2-Dose Regimen of BBV87 Chikungunya Vaccine in Healthy Subjects Aged 12 to 65 years in Latin America and Asia	
● If no criteria were used for grading, or if other metrics were employed, please describe:		
10.4 List and provide frequency of any related or possibly related serious* AEs and well as any severe expected or unexpected AEs observed: (*see Instructions):	None. The results available are for phase I studies. IVI-CHIK-001 (LATAM and Asia study has just started, however no related SAEs have been reported until 24th March 2022. In other words, no SUSARs have occurred.	
10.5 List and provide frequency of any serious, unexpected significantly increased AE or lab abnormality in vaccinee vs. control groups:	None	
● Describe the control group:	N/A	
10.6 List and provide frequency of Adverse Events of Special Interest	None	
10.7 What is the evidence of disease enhancement (if any) in humans?		Antibody-mediated enhancement of CHIKV binding in vitro in primary human monocytes and B cells was observed in the presence of CHIKV specific antibodies. CHIKV antibodies enhanced virus replication in vitro in murine macrophage cell line, RAW264.7 In the presence of sub-optimal neutralizing titers of CHIKV antibodies, higher viral RNA load

Table 1 (continued)

Brighton Collaboration Standardized Template for Collection of Key Information for Benefit-Risk Assessment of Inactivated Viral Vaccines		
10.8 Did a Data Safety Monitoring Board (DSMB) or its equivalent oversee the study?	DSMB monitored safety in phase I clinical trials in India and monitors the ongoing phase II study. DSMB monitors safety in phase II/III study in Latin America (Colombia, Panama, and Asia for the Latin America and Asia study	was observed in 11-day mouse. There is no report of enhancement of the disease in humans due to pre-existing antibodies. ^[39] For the Phase II/III Adaptive Seamless Design, Randomized, Controlled Trial to Evaluate Safety and Immunogenicity of 2-Dose Regimen of BBV87 Chikungunya Vaccine in Healthy Subjects Aged 12 to 65 years In Latin America and Asia, a DSMB oversees the study. that started recruitment on 12Aug22. 5 DSMB meetings are planned and 2 of them include safety go decision to continue with the subsequent parts of the study. <i>Ad hoc</i> meetings will be held if required.
● Did it identify any safety issue of concern?	No	
● If so describe:		
11. Overall Risk Assessment	Information	Comments/ Concerns
11.1 Please summarize key safety issues of concern identified to date, if any: ● how should they be addressed going forward	None	
11.2 What is the potential for causing serious unwanted effects and toxicities in: ● healthy humans? ● immunocompromised humans? ● human neonates, infants, children? ● pregnancy and in the fetus in humans? ● elderly? ● in any other special populations (e.g., institutionalized populations, individuals with associated chronic comorbidity)?	Describe the toxicities None None None None None None	Please rate risk as: none, minimal, low, moderate, high, or unknown

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